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Aerobic TCE degradation by willows and three root colonizing bacterial strains of *B. cepacia*

By

Lauge Clausen

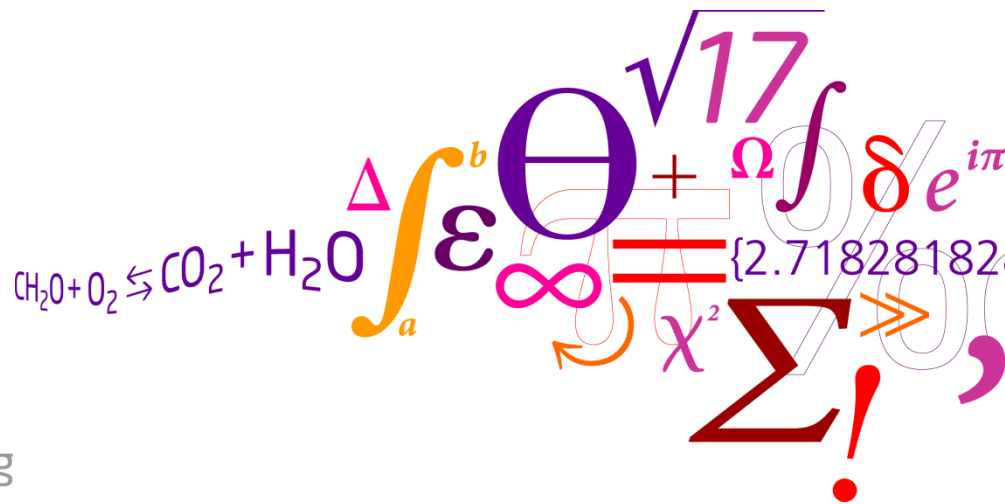
Ulrich Gosewinkel Karlson

Mette Broholm

Stefan Trapp

DTU Environment

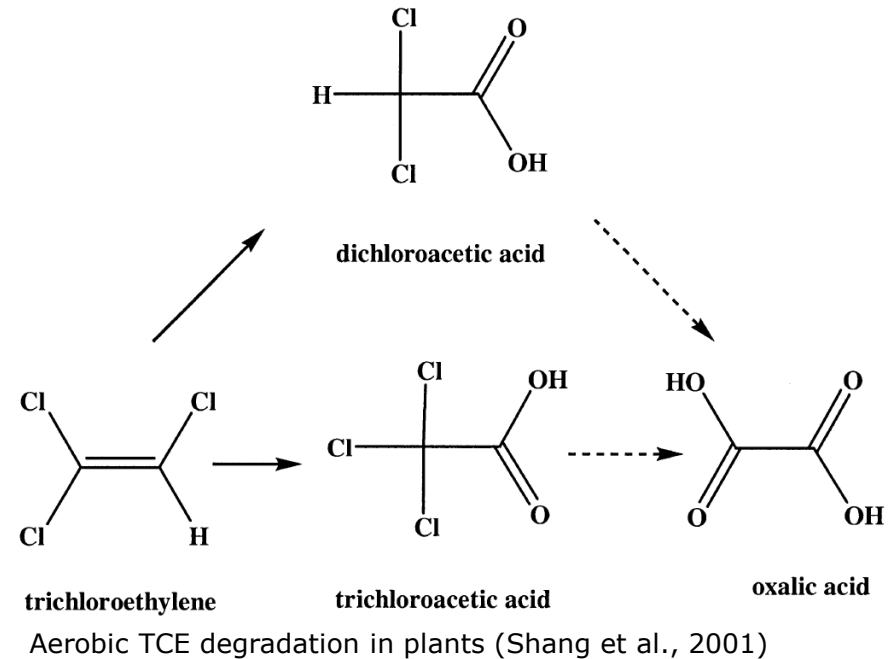
Department of Environmental Engineering



Introduction

Several studies indicate aerobic **Trichloroethylene** (TCE) degradation by plants.

Degradation pathway similar to that of mammals (monooxygenase):



TCE → **trichloroacetic acid** (TCAA) and **dichloroacetic acid** (DCAA) → oxalic acid

But we know very little about the rates...

Test groups

Group	Description	No. of replicates
Control with Cl	Standard Hoagland nutrient solution, no TCE	5
Control no Cl	Modified Hoagland with no Cl, no TCE	5
Low exposure	Start exposure 5 mg TCE/L, no added bacteria	5
High exposure	Start exposure 20 mg TCE/L, no added bacteria	5
Dead	20 mg TCE/L with a dead willow stick, no refill, no added bacteria	4
Control	Start exposure 25 mg TCE/L, no added bacteria	6
301C	Start exposure 25 mg TCE/L, <i>B. cepacia</i> 301C	6
PRI-31	Start exposure 25 mg TCE/L, <i>B. cepacia</i> PRI-31	6
pTOM	Start exposure 25 mg TCE/L, <i>B. cepacia</i> pTOM	6
c301C	<i>B. cepacia</i> 301C, no TCE	3

Temp. ~ 20°C; Light intensity ~ 5000 lux; Humidity ~ 50%

Lab set-up



Burkholderia cepacia (*Pseudomonas cepacia*)

Gram-negative

Rod-shaped

Size: 1.6- 3.2 μm

Degradation of chlorinated compounds (TCE, 2,4,5-trichlorophenoxyacetic acid and more)

(Folsom, 1990)

Colonize plant roots

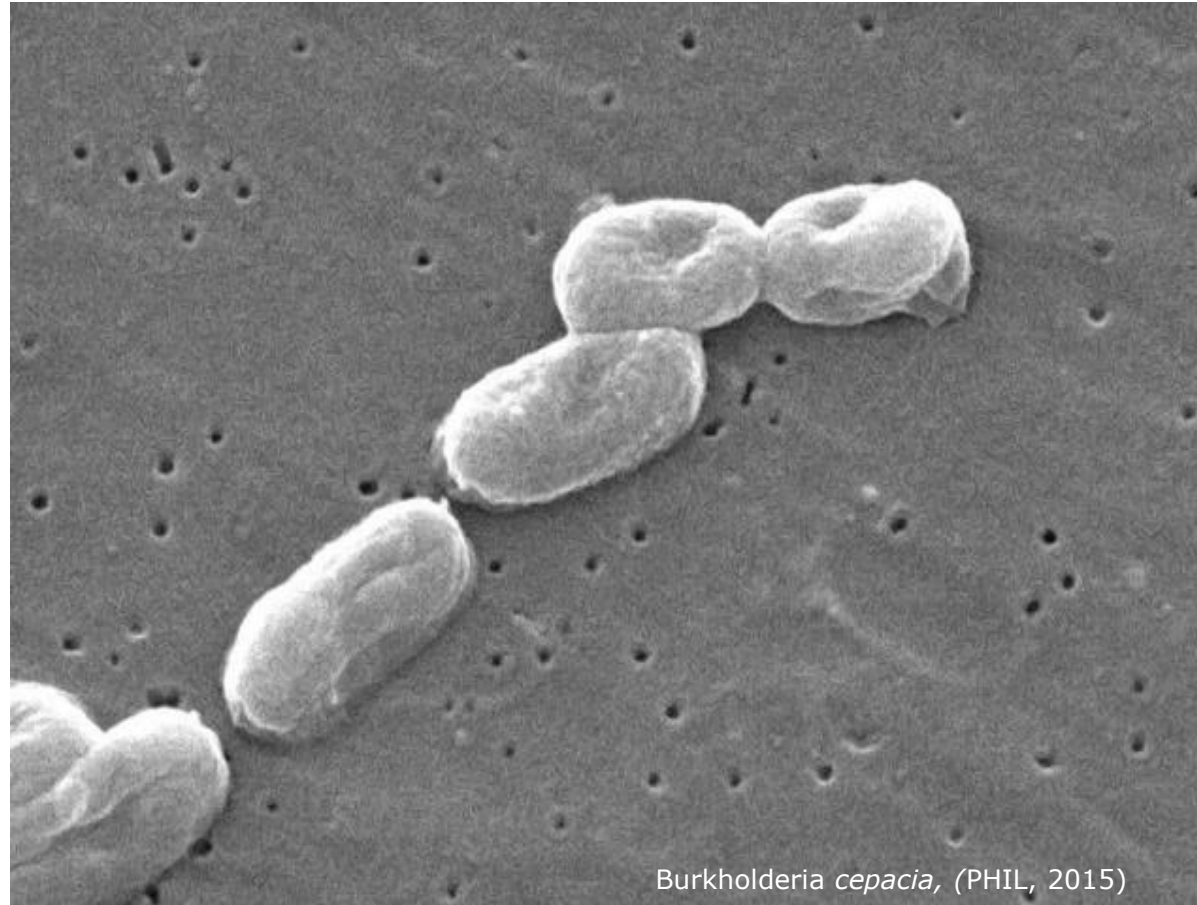
(DMI, 2015)

B. Cepacia strains used:

301C

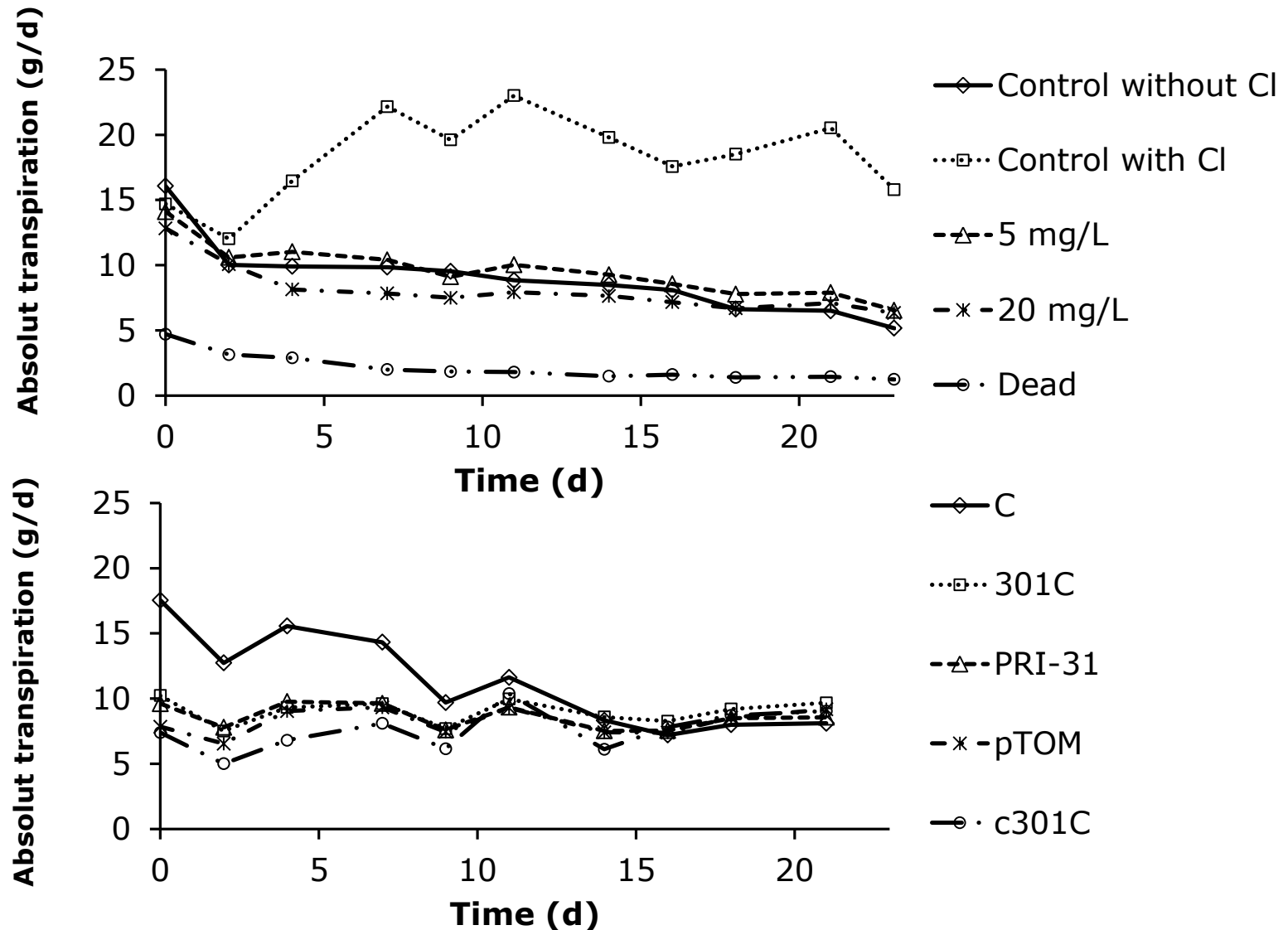
PRI-31

pTOM

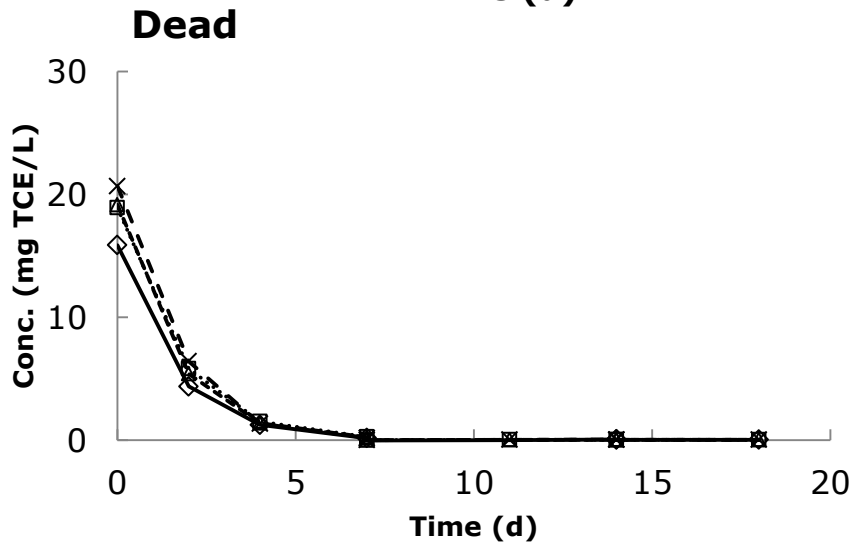
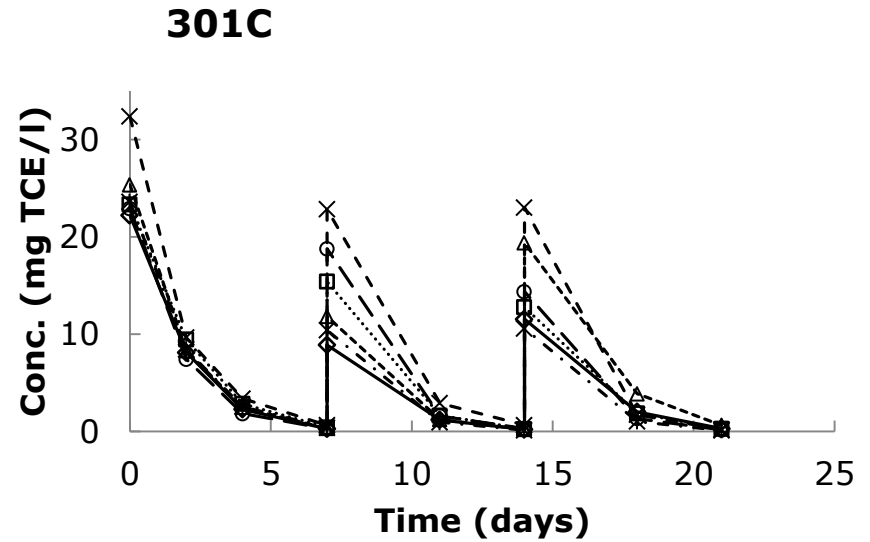
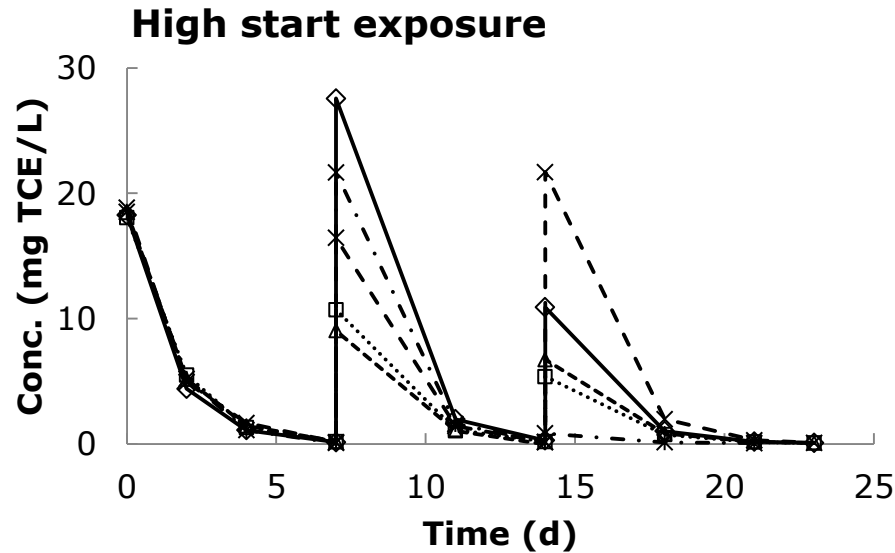


Burkholderia cepacia, (PHIL, 2015)

Transpiration of the willows



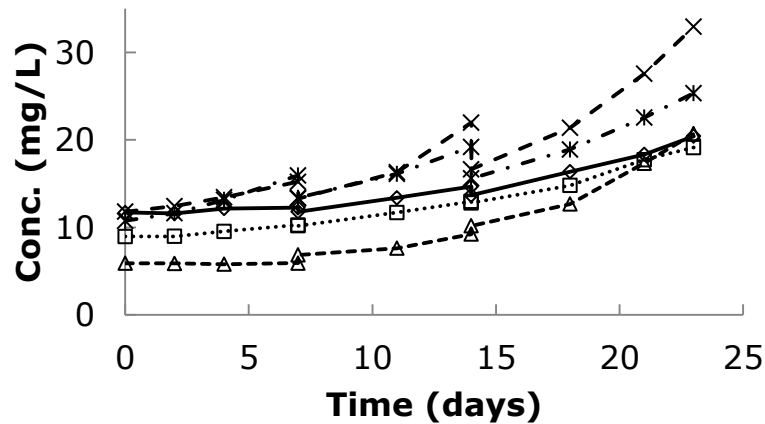
TCE in solution



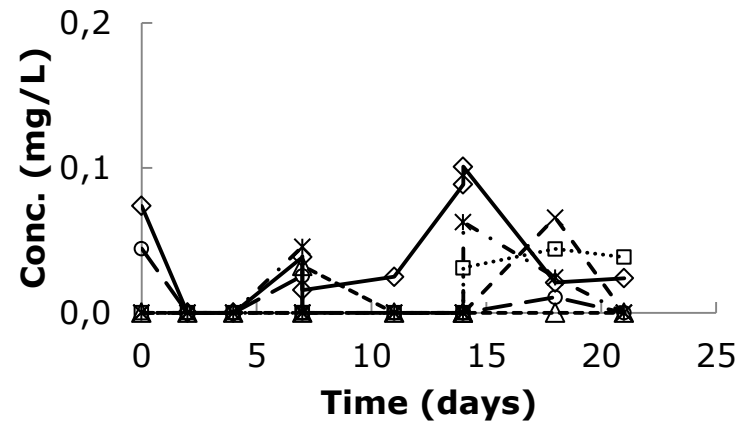
—◇— Rep. 1 ···□··· Rep. 2
 ---△--- Rep. 3 -×- Rep. 4
 -*- Rep. 5

Cl in solution

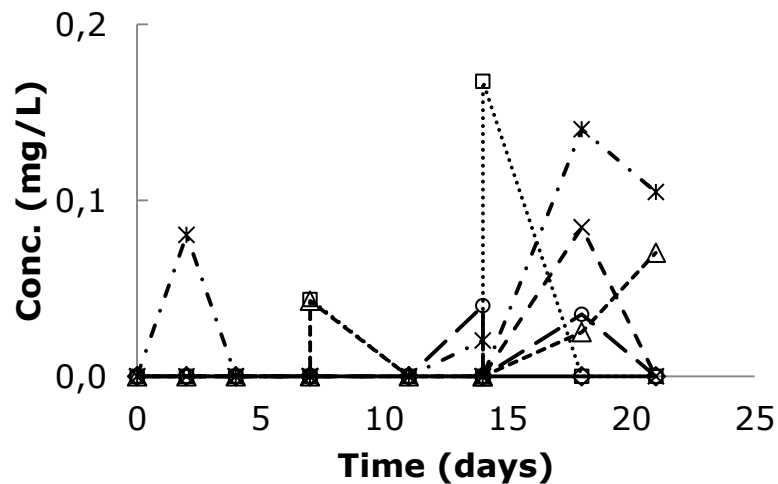
Control (with Cl)



Control (TCE, no added bac.)

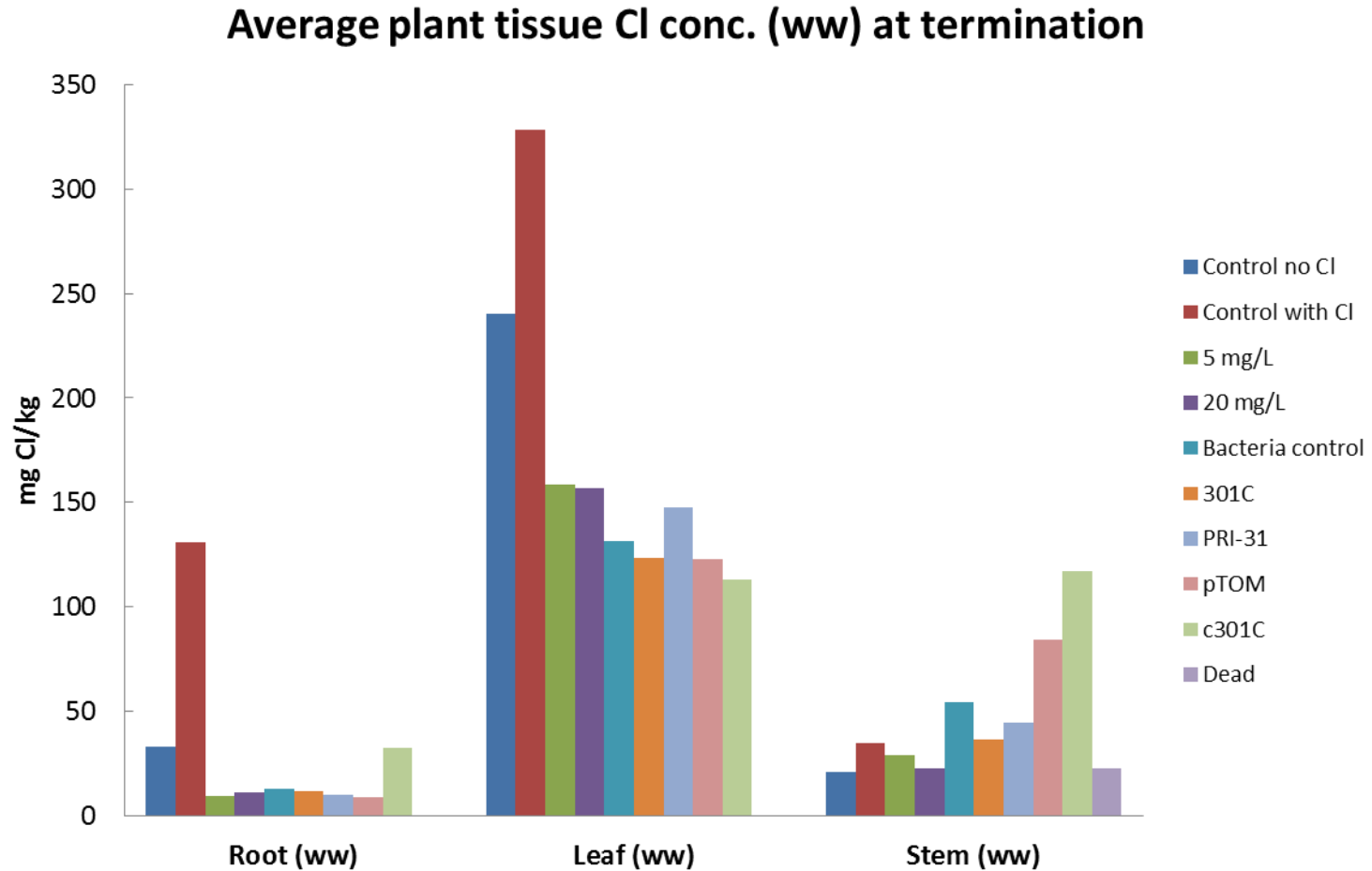


301C



- ◇— Rep. 1
- Rep. 2
- △-- Rep. 3
- ×- Rep. 4
- *- Rep. 5
- Rep. 6

Cl in plant tissue



Cl mass

Mass of Cl (mg) added to the test-systems as TCE, total mass of Cl (mg) in the plants, Cl mass (mg) in solution, total Cl mass present and % distribution of Cl mass in the plant compartments: Root, Leaf and stem, at experiment termination.

Group	Cl added (mg)	Cl in plant (mg)	Cl in sol. (mg)	Tot. Cl (mg)
Control no Cl	0.0 (0.0)	1.9 (0.9)	0.0 (0.0)	1.9 (0.9)
Control with Cl	89.1 (5.7)	4.5 (0.9)	5.6 (1.2)	10.1 (2.1)
5 mg TCE/L	18.9 (6.5)	1.9 (0.9)	0.0 (0.0)	1.9 (0.9)
20 mg TCE/L	20.7 (5.2)	1.3 (0.3)	0.1 (0.1)	1.4 (0.4)
Dead	6.3 (0.7)	0.8 (0.2)	0.0 (0.0)	0.8 (0.2)
Control (TCE, no bac.)	20.9 (3.0)	2.4 (1.0)	0.0 (0.0)	2.4 (1.0)
301C	19.2 (3.4)	1.6 (0.7)	0.0 (0.0)	1.6 (0.7)
PR1-31	18.4 (1.4)	1.8 (1.1)	0.0 (0.0)	1.8 (1.1)
pTOM	17.0 (1.7)	2.5 (2.8)	0.0 (0.0)	2.5 (2.8)
301C, no TCE	0.0 (0.0)	4.9 (6.6)	0.0 (0.0)	4.9 (6.6)

TCE removal rates

First order removal rates (1/day)

Group	rate k (week 1)	rate k (week 2)	rate k (week 3)
5 mg TCE/L	0.77 (0.07)	0.62 (0.06)	0.56 (0.06)
20 mg TCE/L	0.70 (0.05)	0.63 (0.06)	0.55 (0.05)
Dead	0.65 (0.03)	-	-
Control (TCE, no bac.)	0.65 (0.06)	0.58 (0.04)	0.62 (0.06)
301C	0.61 (0.03)	0.58 (0.04)	0.60 (0.07)
PR1-31	0.66 (0.07)	0.60 (0.08)	0.64 (0.09)
pTOM	0.64 (0.05)	0.53 (0.03)	0.59 (0.05)

Values are average of the replicates. Numbers in brackets indicate +/- 95% confidence intervals.

Conclusion

1. Cl conc. in solution increases as the willows take up water
2. No or very little TCE was mineralized to Cl
3. We cannot see significant difference between the TCE removal rates k_{vol} , $k_{vol} + k_{tree}$ and $k_{vol} + k_{tree} + k_{bac}$.

To do...

To improve the study use labelled TCE.

Analysis which will be done:

TCAA (samples stored at -80 °C)

Microbial community of the plant roots

Questions?

References

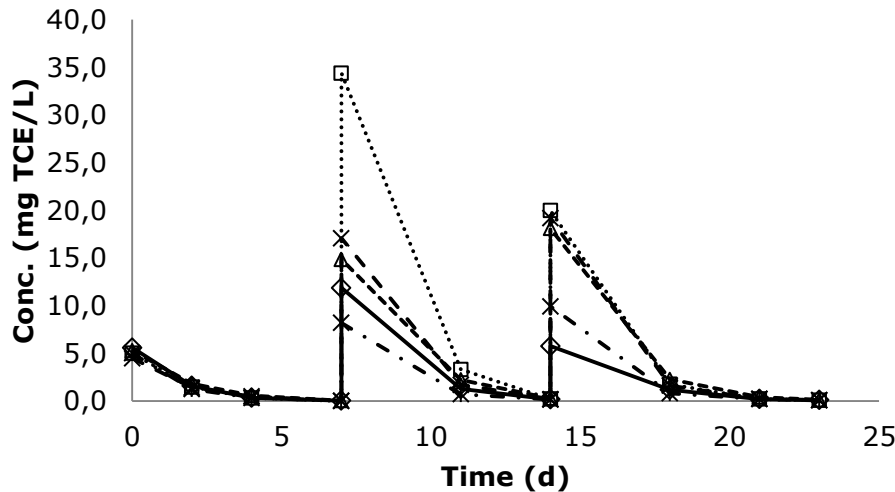
Department of Microbiology and Immunology (DMI), 2015, College of Medicine, University of Illinois; "Genes for 2,4,5- Trichlorophenoxyacetic Acid Metabolism in *B. cepacia* AC1100".

Folsom, B.R., Chapman, P.J. and Pritchard P.H., 1990, Phenol and trichloroethylene degradation by *Pseudomonas cepacia* G4: Kinetics and interactions between substrates. *Appl. Environ. Microbiol.* 56 (5): 1279-1285.

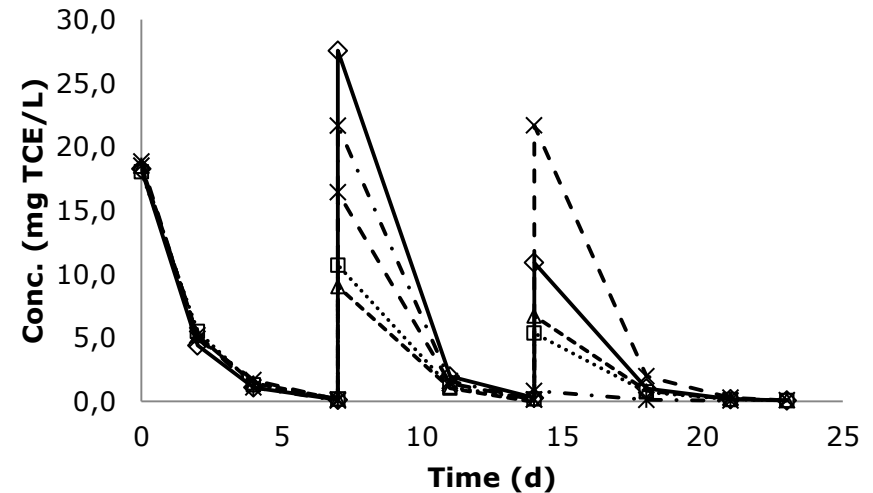
Public Health Image Library (PHIL), 2015, Center for disease control and prevention, link: <http://phil.cdc.gov/phil/details.asp?pid=255> – accessed 15/09/2015

TCE in solution

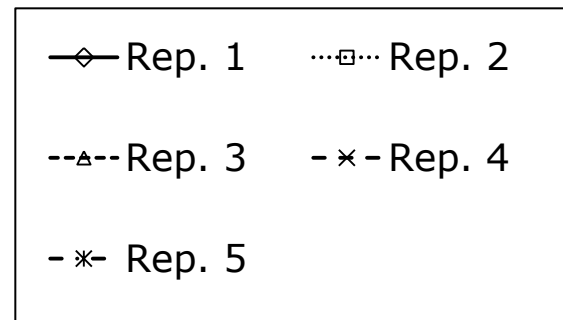
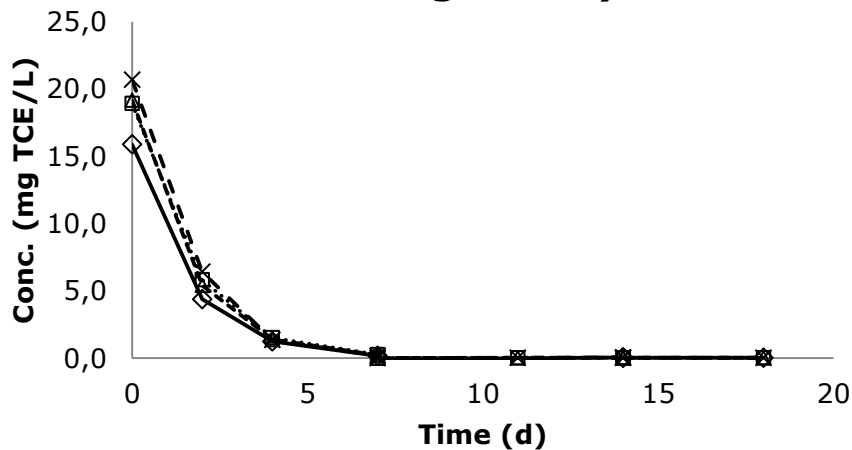
Start exposure 5 mg TCE/L



Start exposure 20 mg TCE/L

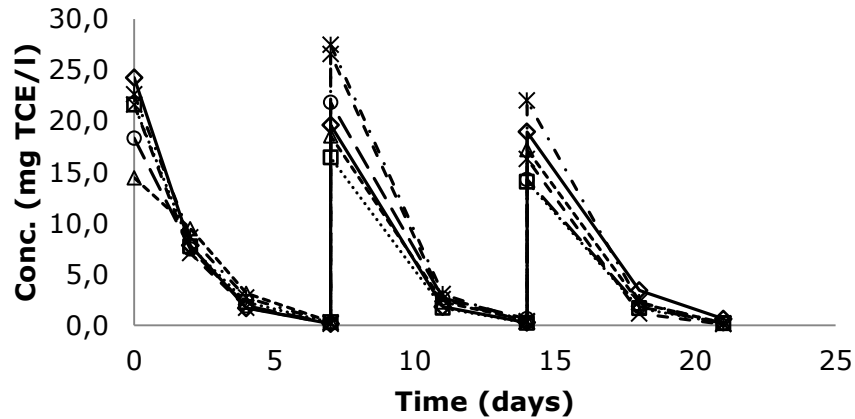


TCE evaporation (Start exposure 20 mg TCE/L)

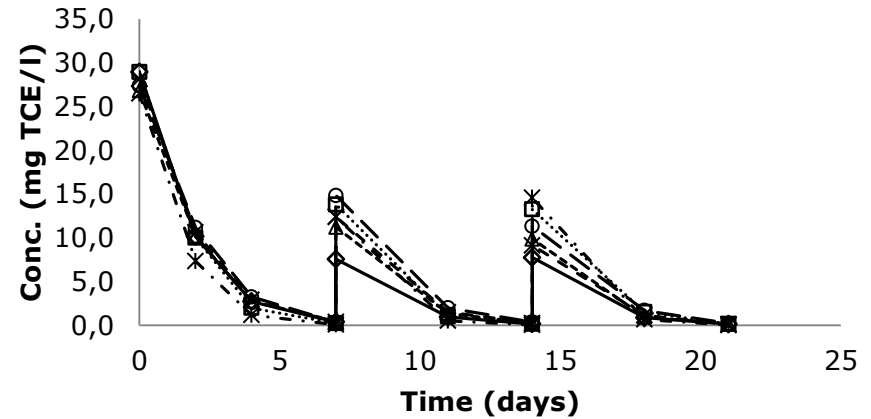


TCE in solution

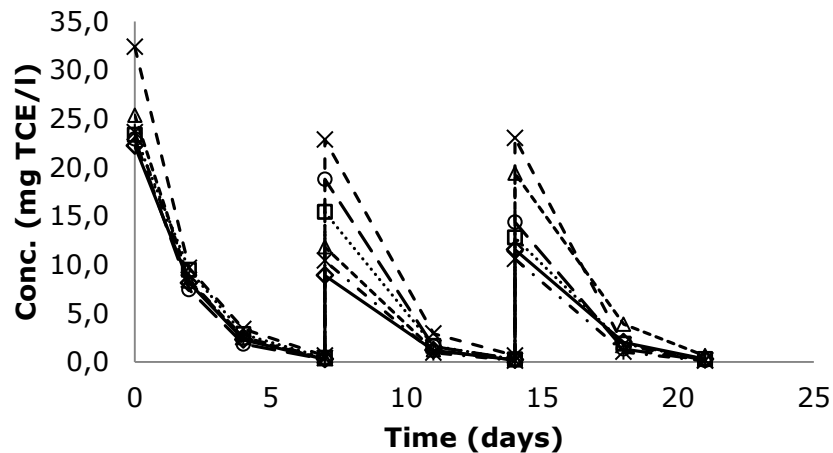
Control (TCE, no added bacteria)



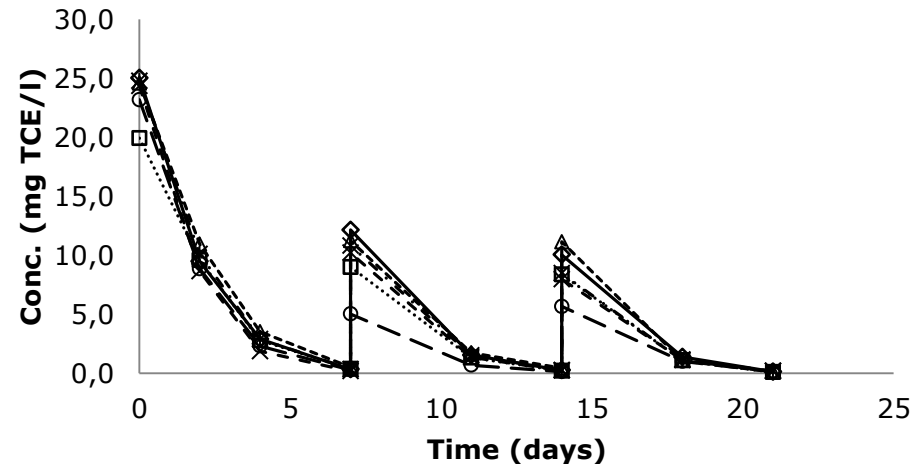
PRI-31



301C



pTOM



—◇— Rep. 1

····□···· Rep. 2

--△-- Rep. 3

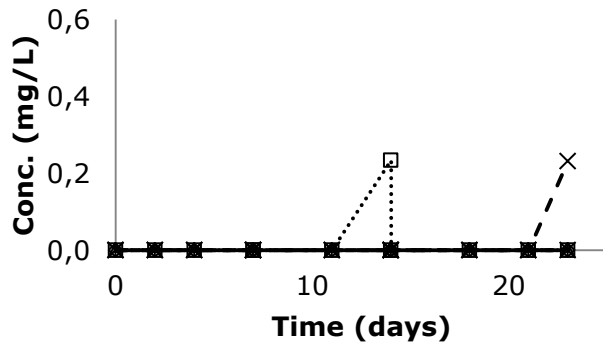
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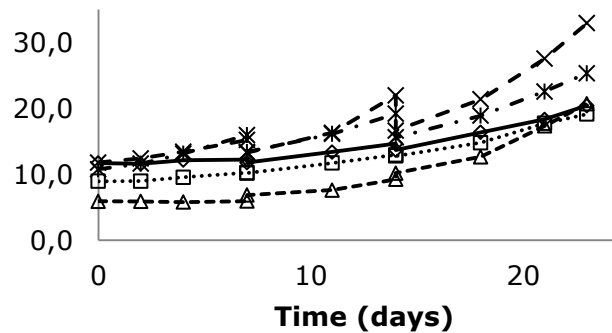
—○— Rep. 6

Cl in solution

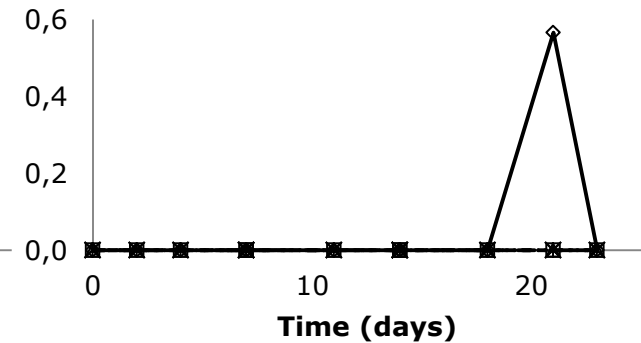
Control (no Cl)



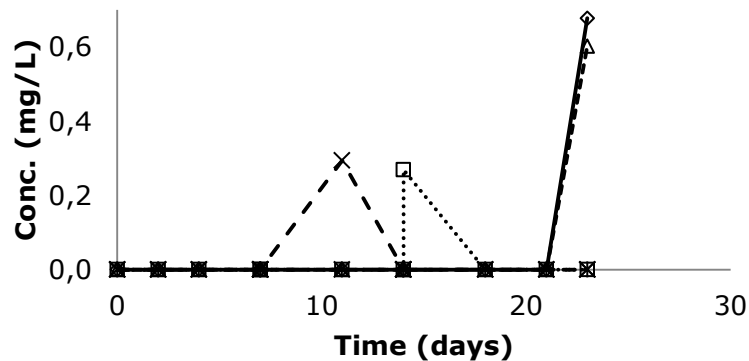
Control (with Cl)



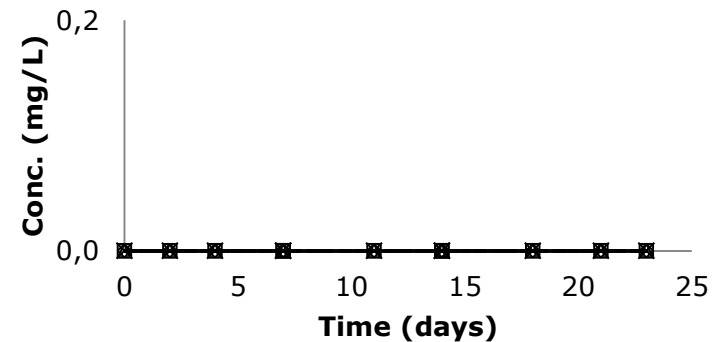
Exposure 5 mg TCE/L



Exposure 20 mg TCE/L



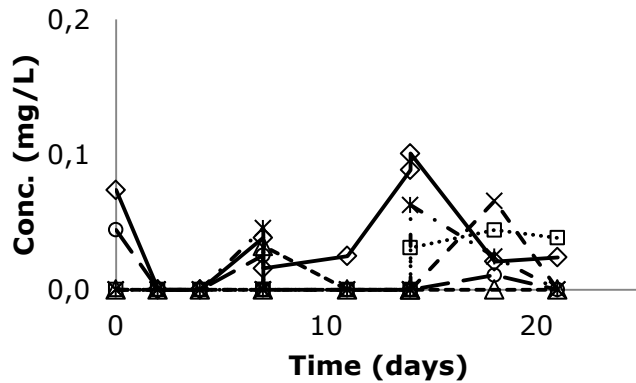
Dead tree sticks



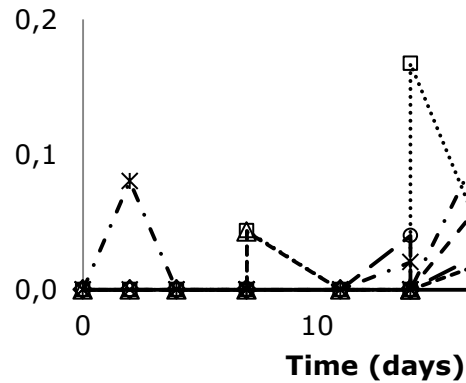
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Cl in solution

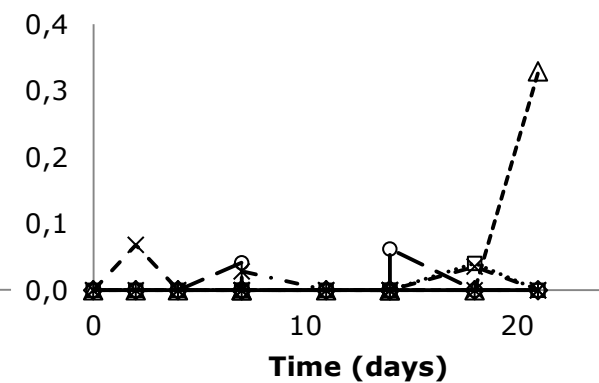
Control (TCE, no added bac.)



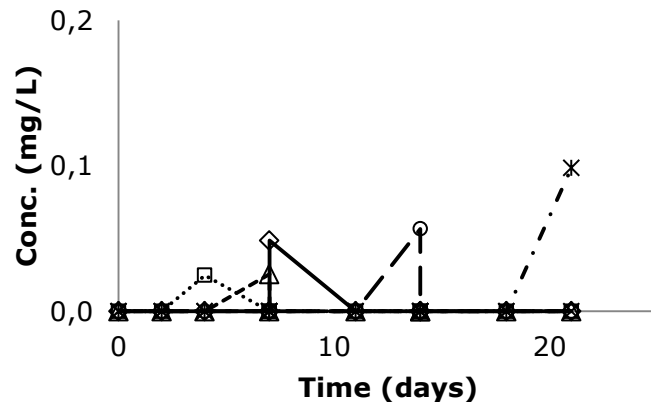
301C



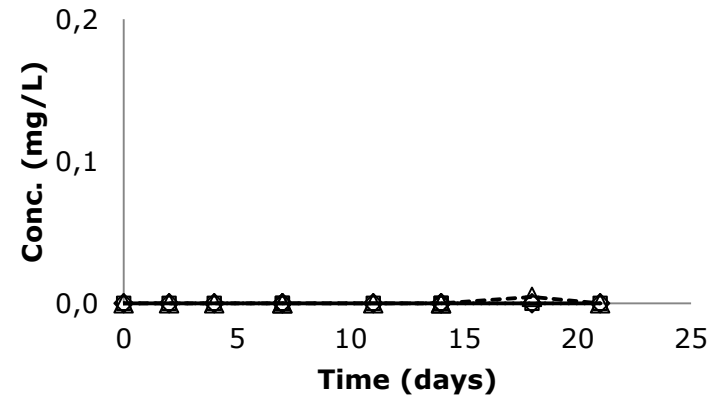
PRI-31



pTOM



301C (no TCE)



—◇— Rep. 1

····□···· Rep. 2

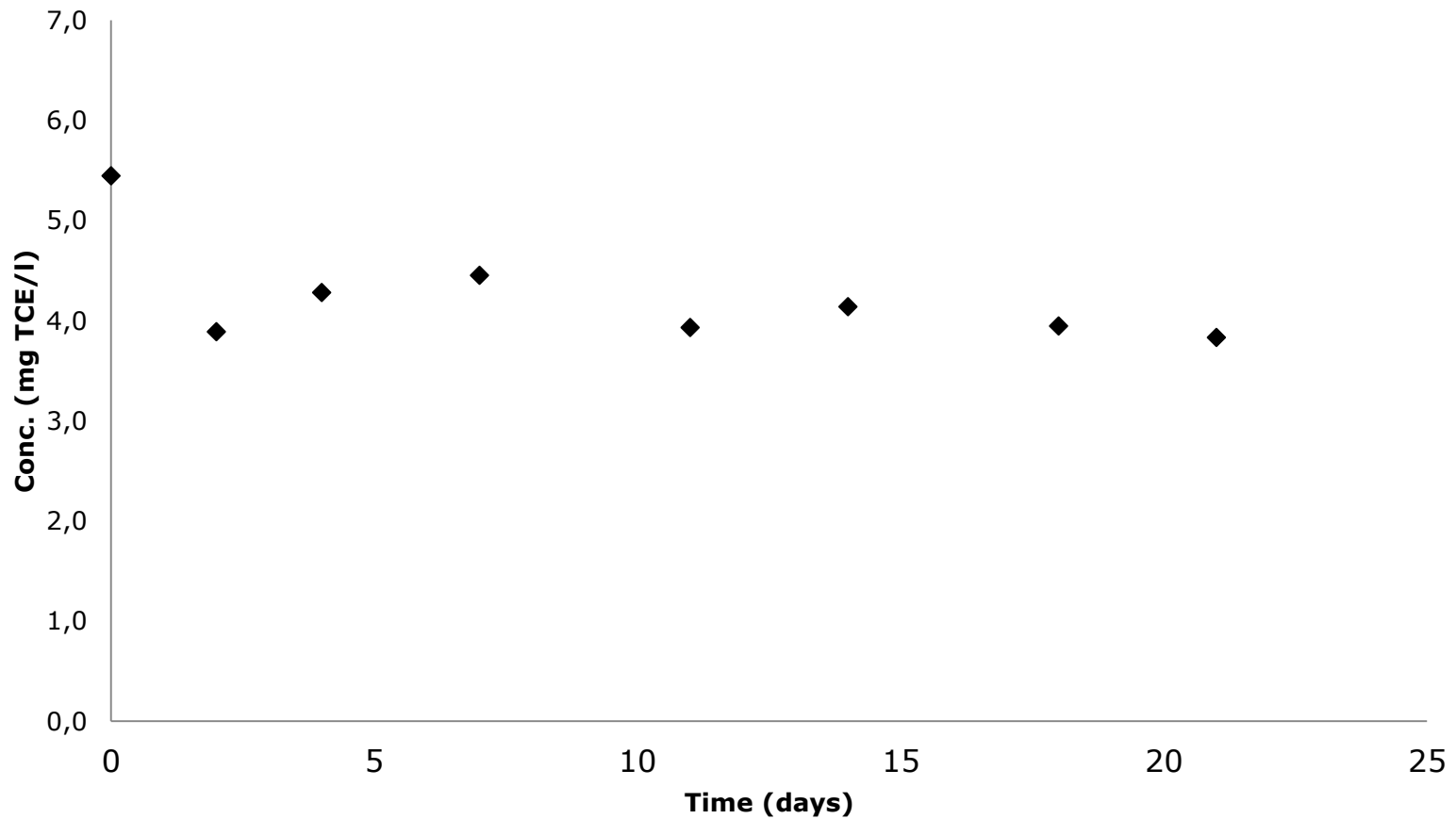
---△--- Rep. 3

- × - Rep. 4

- * - Rep. 5

—○— Rep. 6

TCE in vials with 301C (no trees)



Cl in vials with 301C (no trees)

